IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Shite SEBASTIAN et al	Confirmation No.: 4672
Appl. No.	:	10/576,633	TC/A.U.: 1645
Filed	:	November 14, 2006	Examiner: O. A. OGUNBIYI
For	:	METHODS, PEPTIDES AND BIOSENSORS USEFUL FOR DETECTING A BROAD SPECTRUM OF BACTERIA	

DECLARATION OF MITCHELL C. SANDERS UNDER 37 CFR §1.132

Mail Stop RCE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, MITCHELL C. SANDERS, hereby declare and say:

1. That, I am one of the inventors named in the above-identified patent application;

2. That, I am the Founder and Chief Executive Officer of ECI Biotech, the successor to Expressive Constructs, Inc., one of the orginal assignees of the above-identified patent application;

3. That I have a Ph.D. in Biomedical Sciences from Worcester Polytechnic Institute and have completed postdoctoral training at the Massachusetts Institute of Technology;

4. That, I am familiar with the April 7, 2009 Final Office Action issued by the United States Patent & Trademark Office in connection with the examination of the above-identified patent application; and I am further familiar with the patent examiner's contention in that Final Office Action that the specification of this patent application is not enabling with respect to Claims 1-12 in that application, which claims have accordingly been rejected by the examiner as allegedly being unpatentable under 35 USC §112, First Paragraph;

5. That, I am one of the co-authors of a proposed publication entitled "Rapid Measurement of Protease Activity Prevalent in Bacteria frrm Wounds: A Diagnostic for Total Bioburden", which proposed publication discusses a rapid point-of-care diagnostic, and a copy of which proposed publication is submitted herewith;

6. That, I am also a co-author of a poster entitled "Integration of a Diagnostic Into an Advanced Wound Care Dressing", which poster was presented at the April 26-29, 2009 annual meeting of the Society for Advanced Wound Care, a copy of which poster is also submitted herewith;

7. That, both the above-referenced proposed publication and the above-referenced poster address issues raised by the patent examiner in the April 7, 2009 Final Office Action, namely a) whether the wound bacteria detection method of rejected Claims 1-12 would be "confounded" by the presence in tested wounds or wound fluids of host proteases such as MMPs which may also be present in the wound or wound fluids and which may also cleave the subject peptide substrate described in Claim 1-12; and b) whether the specification examples provide enough information about the wounds being investigated and nature of the bacteria therein to teach the skilled artisan how to practice the bacteria detection method of rejected Claims 1-12;

8. That, I can make the following observations about the operation of the detection method of Claims 1-12, as such claims are being amended herein concurrently with the submission of this Declaration, as well as the following observations about the contents of the proposed publication and of the poster submitted herewith as these items relate to the practice of this claimed detection method:

The detection method of Claims 1-12 as amended herein, relies on the use of a small (10 amino acid) region of the reactive site loop (RSL) of alpha 1 trypsin inhibitor that is placed between an anchor (for example, a 60 μm Tris Acryl or HyperD bead, Pall Life Sciences) and an affinity tag (for example, either blue dye #1 or biotin/poyhistidine dual affinity tag). The steric hindrance of labeling a small peptide with very large bead on the amino terminus and a bulky ring structure of dye or biotin molecule on the carboxy terminus makes it virtually inaccessible to host proteases that are typically much larger (30 kDa) than the active proteolytic form of a bacterial protease. Most active forms of bacterial proteases are <30 kDa, in contrast most MMPs are 30-125 kDa and are often found as dimeric complexes.

Experiments run *in vitro* indicate that a fragment of the RSL referred to as peptide CPI2S does not cross react with MMPs 1,2 9 or 13 or physiologic levels of human neutrophil elastase. This is shown, for example, in Figure 1 for MMPs 1, 2 and 9:

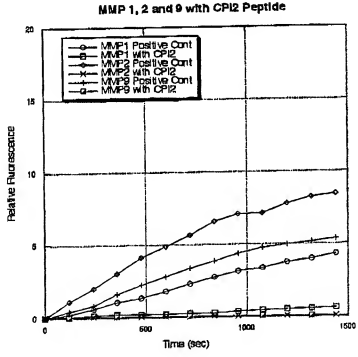

Figure 1

As shown in Figure 1, MMPs 1, 2, and 9 do not cross-react with CPI2 peptide. To generate the Figure 1 data points, fluorescence resonance energy transfer (FRET) assay was performed using 75ng of the indicated MMP, 50μl of MMP standard substrate (positive control) or 5μl CPI2 peptide in a final volume of 200μl. Fluorescence was read every 2 min for 25 min at 355nm emission and 460nm excitation.

In contrast, the CPI2S peptide specifically detects protease from *Staphylococcus aureus, Streptococcus pyogenes, Enterococus faecalis, Pseudomonas aeruginosa, Proteus mirabilis, Serratia marcescens,* and *Acinitobacter baumanii* below the 10^6 CFU per ml level, which the Wound Healing Society considers to be the critical colonization that will lead to an infection. Such detection is discussed in the proposed publication. This proposed publication describes clinical data from UMASS Medical School with 100 chronic wounds that had mixed etiologies: pressure ulcers, diabetic foot ulcers, venous ulcers, and small proportion of post-surgical and post trauma non-healing wounds. For debrided wounds, the broad-spectrum assay described is very accurate with a sensitivity of 93% and a specificity of 80%.

Although the examiner correctly points out that bacterial proteases may be indicative of an infection as taught in the Yolken et al publication, our basic patent, Sanders et al; WO 03/063693, demonstrates, by bioinformatics in which we took the entire genomes from the top 5 wound pathogens and subtracted the genomes from 47 non-pathogenic bacteria that are not normally found in wounds, that there are 132 genes that seem to be specific biomarkers for a wound infection. Many of the 132 genes are virulence factors that are found in pathogenic microorganisms and are not secreted by commensal (non-pathogenic) bacteria that do not infect wounds. The advantage of the method of our Claims 1-12 is that this small fragment of the RSL detects proteases secreted by pathogenic microorganisms.

These proteases are actively involved in tissue destruction of the wound bed that is thought to be the precursor to infection. Antibody based detection methods would not be ideal for detecting the bacteria because they cannot distinguish between live or dead bacteria, and a mix of ten antibodies would be necessary to get the broad-spectrum nature of the RSL fragment used in the method of our Claims 1-12. Because the proteases are labile, they are only present when the bacteria are viable, and thus, are a much more reliable indicator of infection. Our proposed publication describes the biochemical mapping of the specific proteases from bacteria commonly found in wounds and a correlation of these proteases with the cleavage by bacteria both *in vitro* and with clinical samples. These results support the validity of the approach represented by the method of our Claims 1-12. Such a method has led to the development of two new products. The innovation behind these products has been featured on Ivanhoe's Medical breakthroughs (July, 2009) and has been awarded the prestigious NIH accelerator commercialization track (ACT) award in 2009.

The dressing sensor diagnostic is the product application illustrated in the poster. The poster demonstrates that this protease sensor with a food grade dye as the reporter molecule can be incorporated into a dressing sensor that turns blue if an infection is due. The cost of goods for this application is less than three cents, and agreement has been reached with a consumer partner to commercialize our diagnostic into household brands in wound care, baby care, women's health and oral care. Thus, counter to the findings described in the Derocher et al. publication (cited by the examiner) which demonstrated reactivity of MMPs with RSL, we have unequivocally demonstrated that the

RSL sensor in the context of the dressing diagnostic can be quite specific for bacterial proteases. Such a sensor in this context does not cross-react with proteases from blood or human fluid because of the spatial representation of the peptide in the context of a bead anchor and aromatic leaving group (blue dye or biotin/polyhistidine). We very much appreciate that MMPs are activated as part of the pro-inflammatory response in chronic wounds. However we have shown empirically that physiologic levels of these non-bacterial protease are not an issue.

9. That, based on the foregoing observations, and further based on the information in the proposed publication and the poster submitted herewith, it is my opinion and belief that the skilled artisan reading the supporting specification would easily be able to practice the method of Claims 1-12 as now set forth in the instant patent application and, in light of the supporting specification could readily realize and appreciate the diagnostic benefits provided by this wound bacteria detection method.

I, MITCHELL C. SANDERS, further declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements have been made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001, Title 18, of the United States Code, and that such willful false statements may jeopardize the validity of the above-identified application or any patent issued thereon.

Further Declarant sayeth not.

Mitchell C. Sanders

9-8-09
Date

18 U.S.C §1001 Statements or Entries Generally

Whoever, in any matter within the jurisdiction of any department or agency of the United States knowingly and willfully falsifies, conceals, or covers up by any trick, scheme, or device or material fact, or makes any false, fictitious or fraudulent statements or representations, or makes or uses any false writing or document knowing the same to contain any false, fictitious, or fraudulent statement or entry shall be fined not more than $10,000 or imprisoned not more than five years, or both.